

Maitham A. Al-Sammak, Ph.D. Candidate, Environmental Health, Occupational Health, & Toxicology Program, University of Nebraska- Lincoln (UNL)
Dr. Kyle D. Hoagland, School of Natural Resources, UNL
Dr. Daniel D. Snow, Water Center, Water Sciences Laboratory, School of Natural Resources, UNL

ABSTRACT

Photosynthetic microorganisms play an important role in the functioning of aquatic ecosystems, in that they are often among the main primary producers that form the base of aquatic food webs. Several genera of photosynthetic microorganisms are capable of producing toxins that can impact aquatic ecosystems. Cyanobacteria rank among the most important of these; several of its members produce a variety of toxins (termed cyanotoxins), including hepatotoxins and neurotoxins. Here, we focus on the potent neurodegenerative cyanotoxins BMAA (β -Methylamino-L-alanine), which has recently been shown to cause ALS, Alzheimer's, and Parkinson's disease in humans in several parts of the world (e.g., China, the Middle East). Biomagnification of BMAA via the food chain is a recently-studied process of potentially great relevance to both human and animal health. Because cyanobacteria are widespread in freshwater systems and BMAA release is widespread among numerous genera, BMAA could be found in many freshwater systems, resulting in an important human exposure risk.

Thus, the overall objectives of our research are to: (1) develop the methodology necessary to detect this single amino acid, at very low levels, in open water, as well as in macrophyte and fish tissues, (2) better understand the ecological factors that may be involved in the production of BMAA, and (3) ascertain the routes of exposure of BMAA in humans. Here we present findings which address parts of all three objectives, including the first report of BMAA in open fresh water in the U.S.

INTRODUCTION

Non-point source (NPS) nutrient input from agriculture has resulted in accelerated lake aging due to high productivity, a process termed cultural eutrophication. The U.S. EPA has indicated that these NPS inputs is responsible for a majority of water quality impairment nationwide, but especially in the agriculturally dominated Midwestern U.S. One important consequence of eutrophication is the prevalence of blue-green algae, also known as cyanobacteria, in many of the lakes and reservoirs throughout the region. A common outcome of cyanobacterial dominance in the phytoplankton is bad taste and odor (in drinking water supplies), fish kills, and even mortality in cattle and dogs. Human-related problems also include gastrointestinal distress, and contact dermatitis. More recently, BMAA (β -Methylamino-L-alanine) has been identified as a cyanotoxin that likely occurs in both marine and freshwater systems, a potent neurodegenerative toxin that can result in several prevalent neurologic diseases. Few studies have measured multiple algal neurotoxins, and it's important to evaluate the relationships between these compounds.

Thus, the overall goal of the research presented here was to determine if indeed BMAA occurs in lakes and reservoirs in the Midwestern U.S., and ultimately to determine what if any health risk it may pose to the local population in lichens, plants, various protists, or sponges and provide energy for the host. Cyanobacteria produce a wide range of toxins (Table 1). Because these toxins can leech into fresh and marine water systems, cyanobacteria are a growing problem worldwide. One of the cyanotoxins that has recently come into focus is BMAA.

BMAA

BMAA-related Neurodegenerative diseases, include: amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease.

In 2003, it was discovered that cyanobacteria of the genus *Nostoc*, which live as endosymbionts in special roots of cycads, produce BMAA. In 2005, several diverse taxa of cyanobacteria, including free-living and symbiotic species, were found to produce BMAA.

Because BMAA concentrates in the developing reproductive tissue of the cycad, free-living cynaobacteria can produce 0.3ug/g BMAA and 2-37ug/g as symbionts in the coralloid roots of cycad trees.

In one well-known study, it was determined that those members of the Chamorro people on Guam that consumed the cycad seeds suffered from ALS or the Parkinson-dementia complex (PDC) and eventually died from the lethal nature of these diseases. BMAA was also recently found in the brain tissue of nine Canadian Alzheimer patients, thus BMAA-induced neurodegeneration is likely a more widespread phenomenon than originally thought.

This suggests the existence of alternative ecological pathways for the bioaccumulation of BMAA. BMAA was subsequently detected in cyanobacterial blooms and laboratory isolates from marine and freshwater sources from many varied localities including: Iraq, Qatar, Hawaii, China, United Kingdom, South Africa, Netherlands, and Sweden.

Environmental Risk Factors

Some cyanotoxins (e.g., BMAA) pass through host plants to higher organisms and eventually to humans. Two routes have been proposed:

1) Indirect route: Toxins may pass to host plants then other organisms higher in the food chain (e.g., fish, cattle, bats, etc.) and undergo bioaccumulation in the muscles of these organisms. People may ingest this contaminated muscle, introducing the toxins to the human nervous system.

2) Direct route: by swimming or drinking contaminated water (e.g. lakes under health alert) that exposes humans and/or animals to these toxins.



Typical cyanobacterial bloom-related fish kill in a local reservoir



Typical cyanobacterial bloom in a local reservoir



Nostoc, a filamentous, colonial cyanobacterium, forms mucilaginous balls



Anabaena, a filamentous cyanobacterium

| Toxin | Target organ | Activity |
|---------------------|--------------|-------------------------------------|
| Microcystin | Liver | Tumor Promoters |
| Nodularins | Liver | Carcinogenic |
| Cylindrospermopsins | Liver | Genotoxic |
| Anatoxin-a | Nerve System | Depolarizing neuromuscular blockers |
| Anatoxin-a (s) | Nerve System | Inhibits AChE |
| Saxitoxins | Nerve System | Na ⁺ channel blockers |
| BMAA | Nerve System | Unknown |
| Lyngbyatoxin-a | Skin | Inflammatory agents |
| Apysiatoxins | Skin | Inflammatory agents |
| Lipopolysaccharide | G.I.T. | Gastrointestinal irritants |

Table 1. Toxins produced by cyanobacteria

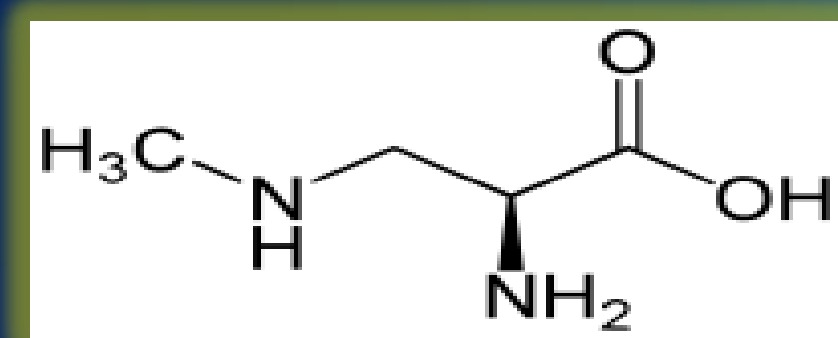


Fig. 1. BMAA chemical structure

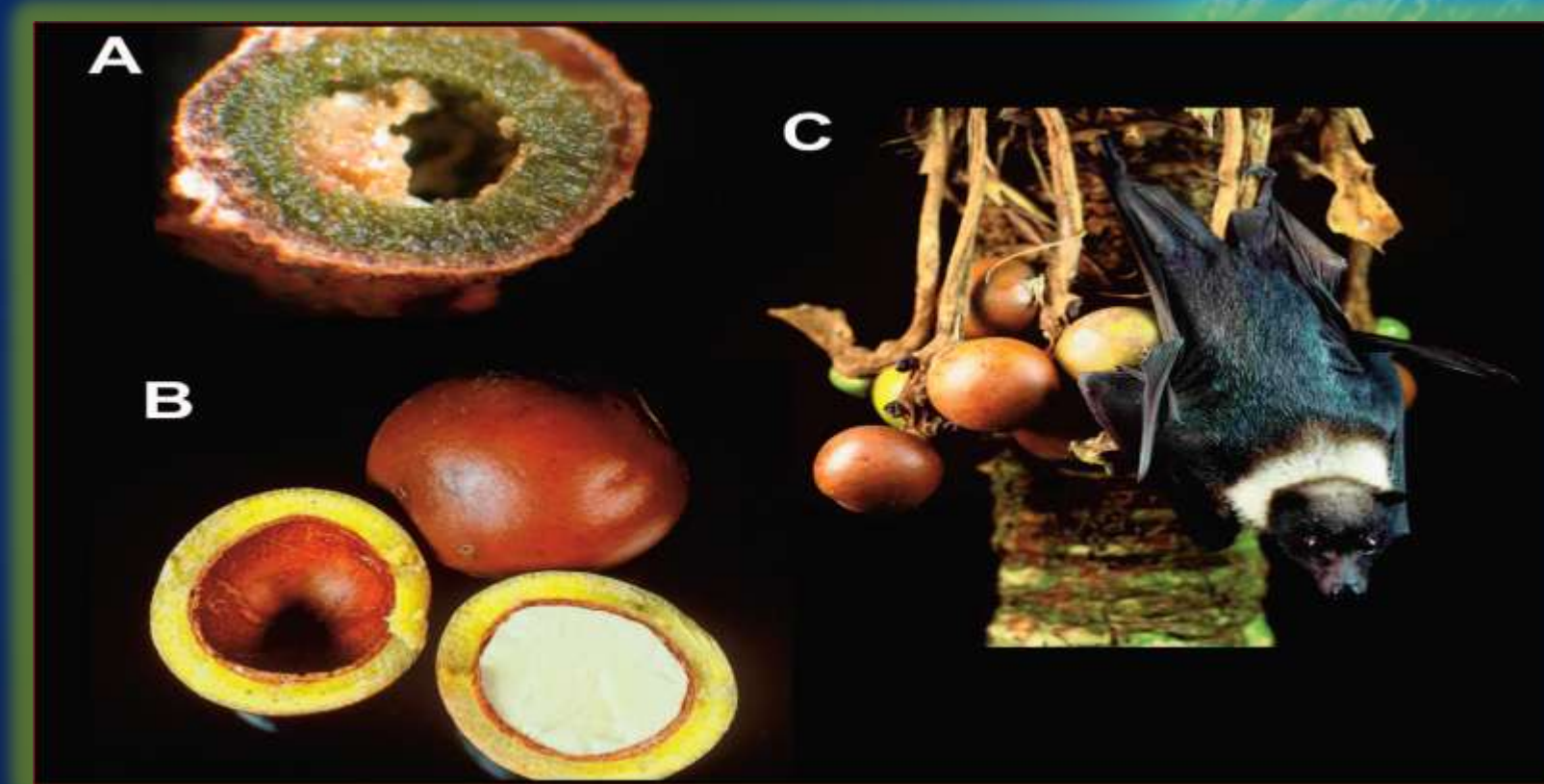


Fig. 2: Biomagnification of BMAA on the island of Guam.
A) Cyanobacteria (*Nostoc* sp.) are beneficial symbionts living in specialized "coralloid" roots of the cycad *Cycas micronesica* (from Cox et al. 2003); B) BMAA is incorporated by the cycad and reaches its highest concentration in the maturing fruits; C) Cycad fruit is a dietary mainstay of the native bat *Pteropus mariannus mariannus*. BMAA is incorporated into proteins and has been reported in high levels in museum specimens. *Continuum: Lifelong Learning Neurol* 2008;14(5)

RESULTS: Water samples data

Table 2. Cyanobacterial neurotoxin levels in open water (grab) samples from 2009 and 2010 (BOAA = L-3-oxalylamino-2-amino-ppropionic acid, and; DABA = DL-2,4-diaminobutyric acid dihydrochloride, an isomer of BMAA). All concentrations are in $\mu\text{g/L}$ (ppb). Detection limits were 5.0 $\mu\text{g/L}$ for BMAA, 4.3 for BOAA, 7.0 for DABA, 6.0 for anatoxin-a, and 5.0 $\mu\text{g/L}$ for microcystin[†].

| Reservoir | Location (NE County) | Dates Sampled* (number of samples) | BMAA 2009/2010 | BOAA 2009/2010 | DABA 2009/2010 | Anatoxin-a 2009/2010 | Microcystin [†] 2009/2010 |
|----------------|----------------------|------------------------------------|----------------|----------------|----------------|----------------------|------------------------------------|
| Holmes Lake | Lancaster | 9.7.09(3)/8.6.10(3) | 6.0/0 | 0/0 | BDL/0 | 5.0/0 | BDL/BDL |
| Pawnee | Lancaster | 10.28.09(2)/8.20.10(3) | 11.3/BDL | BDL/0 | 12.7/0 | 14.4/BDL | 11.0/5.9 |
| Wagon Train | Lancaster | 10.6.09(3)/10.26.10(3) | 0/0 | 0/0 | 0/0 | 0/0 | BDL/BDL |
| Stage Coach | Lancaster | 10.7.09(3)/10.26.10(3) | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| East Twin | Seward | 10.9.09(3)/10.25.10(3) | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| Rockford | Gage | 8.3.09(2)/8.22.10(3) | 24.5/18.3 | BDL/0 | 13.2/13.6 | 8.4/BDL | 44.5/11.9 |
| Kirkman's Cove | Richardson | 8.3.09(2)/8.8.10(3) | BDL/25.3 | 0/4.9 | BDL/14.7 | 35.0/BDL | 14.8/35.0 |
| Swan Creek | Saline | 9.10.09(2)/9.9.10(3) | BDL/BDL | 0/0 | BDL/BDL | BDL/BDL | 6.3/21.0 |
| Conestoga | Lancaster | 10.8.09(03)/8.23.10(3) | 0/0 | 0/0 | 0/0 | 0/0 | 7.8/BDL |
| Willow Creek | Pierce | 8.30.09(2)/10.1.10(3) | 0/12.6 | 0/BDL | 0/21.1 | 0/16.1 | 15.1/35.0 |
| Branched Oak | Lancaster | 9.20.010(2)/9.20.10(3) | 0/0 | 0/0 | 0/0 | 0/BDL | BDL/BDL |
| Bluestem | Lancaster | 9.25.09(2)/9.8.10(3) | 0/0 | 0/0 | 0/BDL | 0/BDL | 18.3/BDL |

o = None Detected; BDL = Below Detection Limit (i.e. the toxin was detected but could not be reliably quantified);
*in MM.DD.YR format; † data from Nebraska Department of Environmental Quality (ELISA kit method); analytical method used: HPLC with fluorescence detector (Al-Sammak in review)

Role of BMAA in ALS and PDC

The ALS-PDC that ravaged Guam three decades ago is considered a variant form of ALS. Victims of the disease exhibited features of any one of three disorders or all of them. These include the nerve degeneration and motor involvement of ALS (Lou Gehrig's Disease), the abnormal gait of PD and the mental degeneration characteristic of dementia of the Alzheimer type. ALS-PDC has previously killed up to 10% of Guam's indigenous Chamorro population. Similar diseases have been observed on the Kii Peninsula in Japan and the southern lowlands of West Papua (Irian Jaya) in Indonesia, each in close proximity to Guam.

The traditional cuisine of the Chamorro people has included fox bats which feed on cycad seeds. It has been suggested that "the plant and animal proteins provide unrecognized reservoirs for the slow release of this toxin".

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LC/MS and HPLC/FD samples results

| Location | Sample name | HPLC/FD in ppb | | | LCQ/MS in ppb | | |
|--------------------------|-------------------|-----------------|-------|------------|----------------|------|------------|
| | | BMAA | DABA | Anatoxin-a | BMAA | DABA | Anatoxin-a |
| Holmes Lake 2009 | Lake water | 6 | 6.9 | 5 | 15.6 | 18.4 | 19.7 |
| Kirkman's Cove Lake 2009 | Lake water | 2.1 | 3.9 | 35 | 6.52 | 10.8 | 35.7 |
| Pawnee Lake 2009 | Lake water | 11.3 | 12.7 | 14.4 | 39.6 | 37.0 | 29.8 |
| Willow Creek 2010 | Lake water | 12.6 | 21.14 | 16.1 | 23.6 | 32.3 | 30.1 |
| Rockford Lake 2009 | Lake water | 24.5 | 13.2 | 8.4 | 27.5 | 30.0 | 33.2 |
| Swan Creek 2009 | Lake water | 1.8 | 3 | 1.2 | 10.9 | 18.9 | 5.2 |
| Pawnee Lake 2010 | Lake water | 4.2 | ND | 3.04 | 5.01 | ND | 13.5 |
| Kirkman's Cove 2010 | Lake water | 25.3 | 14.7 | 6 | 29.0 | 35.1 | 18.6 |
| Rockford Lake 2010 | Lake water | 18.3 | 13.6 | 4.8 | 24.0 | 33.1 | 22.6 |
| Location | Sample name | HPLC/FD in ppm* | | | LCQ/MS in ppm* | | |
| Swan Creek 2010 | Aquatic Plant-faa | 2.61 | 1.96 | 0.38 | 2.38 | 1.28 | 0.711 |
| Pawnee Lake 2010 | Aquatic Plant-faa | 3.51 | ND | 0.36 | 3.41 | ND | 1.45 |
| Pawnee Lake 2010 | Aquatic Plant-baa | 6.56 | 2.11 | 2.47 | 19.0 | 1.85 | 3.23 |
| Holmes Lake 2010 | Aquatic Plant-baa | 4.5 | 1.61 | 4.44 | 2.69 | 1.09 | 7.92 |
| Kirkman's Cove Lake 2009 | Aquatic Plant-baa | 12.7 | 2.59 | 8.05 | 15.25 | 11.7 | 10.18 |
| Pawnee Lake 2009 | Shad - faa | 0.196 | ND | ND | 0.18 | ND | ND |
| Rockford Lake 2009 | Catfish - baa | 0.32 | 0.28 | ND | ND | ND | ND |
| Rockford Lake 2009 | Catfish - faa | 0.25 | ND | ND | 0.55 | ND | ND |
| Rockford Lake 2010 | Carp - baa | 2.57 | 1.53 | ND | 4.13 | 5.07 | ND |
| Kirkman's Cove 2010 | Carp - faa | 0.102 | 0.158 | ND | 0.23 | 0.21 | ND |

faa: Free amino acid ; baa: Bounded amino acid; *ppm= $\mu\text{g/gm}$

RESULTS: Highlights

- BMAA levels ranged from 6.0 $\mu\text{g/L}$ in Holmes reservoir, to 25.3 $\mu\text{g/L}$ in Kirkman's Cove Reservoir
- BMAA was detected in 6 of the 10 reservoirs with histories of cyanobacterial blooms
- BOAA and DABA were detected in lower concentrations, with BOAA found in measureable quantities in just one reservoir (Kirkman's Cove, at 4.9 $\mu\text{g/L}$), whereas DABA was found in 7 sites, ranging from 12.7 $\mu\text{g/L}$ in Pawnee Reservoir to 21.1 $\mu\text{g/L}$ in Willow Creek Reservoir
- Anatoxin-a, also a powerful neurotoxin, was detected in 8 of the 10 reservoirs with a predominance of cyanotoxins; microcystin was found in all but the control reservoirs, with concentrations ranging from 5.9 to 44.5 $\mu\text{g/L}$; a general lack of a strong correlation between the level of microcystin and BMAA is not surprising in light of the likely differences in the fate and transport, and potential for bioaccumulation of these two neuro-cyanotoxins
- In instances where microcystin was found at higher concentrations (>20 $\mu\text{g/L}$), BMAA also typically occurred (i.e. in 3 of 4 samples from 2009-10) at levels well above the detection limit, indicating that there is a reasonable probability that reservoirs for which a Health Alerts are issued are likely to also have detectable levels and in some cases significant concentrations of BMAA present in the water column.

SUMMARY & FUTURE RESEARCH

As a result of agricultural inputs, cyanobacterial blooms are a common occurrence in Midwestern water bodies. BMAA is a neurotoxin produced by all cyanobacteria tested thus far in both freshwater and marine environments. It has the potential to bioaccumulate in many different species including fish, plants, land animals, and in humans, leading to severe neurodegenerative diseases, including ALS, Alzheimer's and Parkinson's Disease

There is still much to be learned about BMAA and its role in the environment. Suggestions for future studies include the assessment of BMAA biomagnification potentials in other countries outside of Guam and the risk factors associated with both acute and chronic exposures to humans